

1	601	103.0	110	20	AAVZ8877	Recombinant RACOR1
2	601	100.0	111	20	AAVZ8878	Recombinant Mel(-)
3	597	99.3	110	20	AAVZ8872	Rana catesbeiana C
4	597	99.3	111	20	AAVZ8873	Recombinant Mel(-)
5	591	93.3	110	20	AAVZ8874	Recombinant RACOR1
6	591	93.3	111	20	AAVZ8876	Recombinant Mel(-)
7	586.5	97.6	111	20	AAVY3321	Frog lectin protein
8	282.5	47.0	104	18	AAV06544	Antitumour protein
9	280.5	46.7	104	18	AAVZ8870	Recombinant RACOR1
10	280.5	46.7	105	20	AAVZ8871	Recombinant Mel(-)

PA (USSH) US DEPT HEALTH & HUMAN SERVICES.

RESULT 1

PI Newton DL, Rybak SM;
XX
XX MPI: 1999-610847/52.
DR N-PSDB; AAZ08134.
XX
PT New recombinant ribonucleases, used for killing target cells, e.g. for
PT treating cancers, viral infections or autoimmune diseases
XX
PS Claim 22; Page 67; 71pp; English.
XX
CC The present sequence is a recombinant Rana catesbeiana oocyte
CC ribonuclease (RacOR1) protein with Gln1Ser. Carboxy terminal end of
CC recombinant RacOR1 has a covalently bound ligand binding moiety, which
CC can be a LL2 antibody directed against CD22 on cancerous B cells or
CC human chorionic gonadotropin (hCG) effective against Kaposi's sarcoma
CC cells. Recombinant ribonucleases can be expressed in bacteria without an
CC N-terminal methionine due to the presence of a signal peptide that is
CC cleaved by bacteria. The soluble expression of ribonuclease allows the
CC cytotoxic to be fused in-frame with ligand binding moieties to form
CC cytotoxic fusion proteins. They can be used for treatment of cancer and
CC autoimmune diseases.
XX
SQ Sequence 110 AA;

Query Match 100.0%; Score 601; DB 20; Length 110;
Best Local Similarity 100.0%; Pred. No. 3.1e-61;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Caps 0;

QY 1 SNNATFOQKHIIPTPIICNTIMDNNIYIVGGCKRVNTFISSATVKAICTGVINMNVL 60
DB 1 SNNATFOQKHIIPTPIICNTIMDNNIYIVGGCKRVNTFISSATVKAICTGVINMNVL 60
QY 61 STTRFOLNCTRTSITPRCPYSSRTETNYICVCKENQYVPHFAGIGRCP 110
DB 61 STTRFOLNCTRTSITPRCPYSSRTETNYICVCKENQYVPHFAGIGRCP 110

RESULT 2
AAZ28878
ID AAY28878 standard; Protein; 111 AA.
XX
AC AAY28878;
XX
DT 25-JAN-2000 (first entry)
XX
DE Recombinant Met(-1) RacOR1 Gln1Ser amino acid sequence.
XX
KW Recombinant Met(-1) Rana catesbeiana oocyte ribonuclease Gln1Ser; RacOR1;
KW covalently bound; LL2 antibody; ligand binding moiety; cancerous B cell;
KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
KW recombinant ribonuclease; cytotoxic fusion protein; cancer; bullfrog;
KW CD22; RNase; autoimmune disease.
XX
OS Rana catesbeiana.
OS Synthetic.
OS
FH Key Location/Qualifiers
FT MISC-difference 1 /note= "Met not found in wild type RacOR1"
FT MISC-difference 2 /note= "Wild type Gln replaced with Ser"
FT
XX
PN MO9950398-A2.
XX
PD 07-OCT-1999.
XX
PF 26-MAR-1999; 99WO-US06641.
XX
PR 27-MAR-1998; 98US-0079751.
XX
PA (USSH) US DEPT HEALTH & HUMAN SERVICES.
XX
PI Newton DL, Rybak SM;

XX
XX MPI: 1999-610847/52.
DR N-PSDB; AAZ08135.
XX
PT New recombinant ribonucleases, used for killing target cells, e.g. for
PT treating cancers, viral infections or autoimmune diseases
XX
PS Claim 22; Page 68; 71pp; English.
XX
CC The present sequence is a recombinant Rana catesbeiana ribonuclease
CC (RacOR1) protein with Met at position 1 and Gln2Ser. Carboxy terminal end
CC of recombinant RacOR1 has a covalently bound ligand binding moiety, which
CC can be a LL2 antibody directed against CD22 on cancerous B cells or human
CC chorionic gonadotropin (hCG) effective against Kaposi's sarcoma cells.
CC Recombinant ribonucleases can be expressed in bacteria without an N-
CC terminal methionine due to the presence of a signal peptide that is
CC cleaved by bacteria. The soluble expression of ribonuclease allows the
CC cytotoxic to be fused in-frame with ligand binding moieties to form
CC cytotoxic fusion proteins. They can be used for treatment of cancer and
CC autoimmune diseases.
XX
SQ Sequence 111 AA;

Query Match 100.0%; Score 601; DB 20; Length 111;
Best Local Similarity 100.0%; Pred. No. 3.1e-61;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Caps 0;

QY 1 SNNATFOQKHIIPTPIICNTIMDNNIYIVGGCKRVNTFISSATVKAICTGVINMNVL 60
DB 2 SNNATFOQKHIIPTPIICNTIMDNNIYIVGGCKRVNTFISSATVKAICTGVINMNVL 61
QY 61 STTRFOLNCTRTSITPRCPYSSRTETNYICVCKENQYVPHFAGIGRCP 110
DB 62 STTRFOLNCTRTSITPRCPYSSRTETNYICVCKENQYVPHFAGIGRCP 111

RESULT 3
AAZ28872
ID AAY28872 standard; Protein; 110 AA.
XX
AC AAY28872;
XX
DT 25-JAN-2000 (first entry)
XX
DE Rana catesbeiana oocyte ribonuclease (RacOR1) amino acid sequence.
XX
KW Rana catesbeiana oocyte ribonuclease; RacOR1; covalently bound; CD22;
KW LL2 antibody; ligand binding moiety; cancerous B cell; Kaposi's Sarcoma;
KW human chorionic gonadotropin; hCG; recombinant ribonuclease; bullfrog;
KW signal peptide; cytotoxic fusion protein; cancer; autoimmune disease;
KW RNase.
XX
OS Rana catesbeiana.
OS Synthetic.
OS
FH Key Location/Qualifiers
FT MISC-difference 1 /note= "Met not found in wild type RacOR1"
FT MISC-difference 2 /note= "Wild type Gln replaced with Ser"
FT
XX
PN MO9950398-A2.
XX
PD 07-OCT-1999.
XX
PF 26-MAR-1999; 99WO-US06641.
XX
PR 27-MAR-1998; 98US-0079751.
XX
PA (USSH) US DEPT HEALTH & HUMAN SERVICES.
XX
PI Newton DL, Rybak SM;
XX
PT New recombinant ribonucleases, used for killing target cells, e.g. for
PT treating cancers, viral infections or autoimmune diseases
XX

PS Claim 22; Page 62; 71pp; English;
 CC The present sequence is a Rana catesbeiana oocyte ribonuclease (RacOR1)
 CC protein encoded by a cDNA modified for expression in E. coli. Carboxy
 CC terminal end of RacOR1 has a covalently bound ligand binding moiety,
 CC which can be a LL2 antibody directed against CD22 on cancerous B cells
 CC or human chorionic gonadotropin (hCG) effective against Kaposi's
 CC Sarcoma cells. Recombinant ribonucleases can be expressed in bacteria
 CC without an N-terminal methionine due to the presence of a signal peptide
 CC that is cleaved by bacteria. The soluble expression of ribonuclease
 CC allows the proteins to be fused in-frame with ligand binding moieties to
 CC form cytotoxic fusion proteins. They can be used for treatment of cancer
 CC and autoimmune diseases.
 CC
 SQ Sequence 110 AA;
 Query Match 99.3%; Score 597; DB 20; Length 110;
 Best Local Similarity 100.0%; Pred. No. 8.9e-61;
 Matches 109; Conservative 0; Mismatches 0; Indels 0; Gaps 0;
 OY 2 NMAATFOOKHIIIMPICNTIMDNNIYIVGGCKRVNFTFISSATTVAICGVMNVLS 61
 Db 2 NMAATFOOKHIIIMPICNTIMDNNIYIVGGCKRVNFTFISSATTVAICGVMNVLS 61
 OY 62 TTRFOLMTCTRTSITPRCPYSSRTETNYICVKCENQPVHFAIGRCP 110
 Db 62 TTRFOLMTCTRTSITPRCPYSSRTETNYICVKCENQPVHFAIGRCP 110
 RESULT 4
 AAY28873 ID AAY28873 standard; Protein: 111 AA.
 AC AAY28873;
 XX 25-JAN-2000 (first entry)
 DT
 XX
 DE Recombinant Met(-1) RacOR1.
 XX
 KW Recombinant Met(-1) Rana catesbeiana oocyte ribonuclease; RacOR1; CD22;
 KW covalently bound; LL2 antibody; ligand binding moiety; cancerous B cell;
 KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
 KW recombinant ribonuclease; cytotoxic fusion protein; cancer; bullfrog;
 KW RNase; autoimmune disease.
 XX
 OS Rana catesbeiana.
 OS Synthetic.
 XX
 FH Key Location/Qualifiers
 FT Misc-difference 1 /note= "Met not found in wild type RacOR1"
 FT
 XX
 PN MO9950398-A2.
 XX
 PD 07-OCT-1999.
 XX
 PF 26-MAR-1999; 99WO-US06641.
 XX
 PR 27-MAR-1998; 98US-0079751.
 XX
 PA (USSH) US DEPT HEALTH & HUMAN SERVICES.
 XX
 PI Newton DL, Rybak SM;
 XX
 DR WPI: 1999-610847/52.
 DR N-PSDB: AA208131.
 XX
 PT New recombinant ribonucleases, used for killing target cells, e.g. for
 PT treating cancers, viral infections or autoimmune diseases
 XX
 PS Claim 22; Page 63; 71pp; English.
 CC The present sequence is a recombinant Rana catesbeiana oocyte

CC ribonuclease (RacOR1) protein with Met at position 1. Carboxy terminal
 CC end of recombinant RacOR1 has a covalently bound ligand binding moiety,
 CC which can be a LL2 antibody directed against CD22 on cancerous B cells or
 CC human chorionic gonadotropin (hCG) effective against Kaposi's sarcoma
 CC cells. Recombinant ribonucleases can be expressed in bacteria without an
 CC N-terminal methionine due to the presence of a signal peptide that is
 CC cleaved by bacteria. The soluble expression of ribonuclease allows the
 CC proteins to be fused in-frame with ligand binding moieties to form
 CC cytotoxic fusion proteins. They can be used for treatment of cancer and
 CC autoimmune diseases.
 CC
 SQ Sequence 111 AA;
 Query Match 99.3%; Score 597; DB 20; Length 111;
 Best Local Similarity 100.0%; Pred. No. 9e-61;
 Matches 109; Conservative 0; Mismatches 0; Indels 0; Gaps 0;
 OY 2 NMAATFOOKHIIIMPICNTIMDNNIYIVGGCKRVNFTFISSATTVAICGVMNVLS 61
 Db 3 NMAATFOOKHIIIMPICNTIMDNNIYIVGGCKRVNFTFISSATTVAICGVMNVLS 62
 OY 62 TTRFOLMTCTRTSITPRCPYSSRTETNYICVKCENQPVHFAIGRCP 110
 Db 63 TTRFOLMTCTRTSITPRCPYSSRTETNYICVKCENQPVHFAIGRCP 111
 RESULT 5
 AAY28874 ID AAY28874 standard; Protein: 110 AA.
 AC AAY28874;
 XX 25-JAN-2000 (first entry)
 DT
 XX
 DE Recombinant RacOR1 Met22Leu Met57Leu amino acid sequence.
 XX
 KW Recombinant Rana catesbeiana oocyte ribonuclease; covalently bound;
 KW RacOR1 Met22Leu Met57Leu; LL2 antibody; ligand binding moiety; CD22;
 KW cancerous B cell; Kaposi's sarcoma; human chorionic gonadotropin; hCG;
 KW signal peptide; recombinant ribonuclease; cytotoxic fusion protein;
 KW cancer; bullfrog; RNase; autoimmune disease.
 XX
 OS Rana catesbeiana.
 OS Synthetic.
 XX
 FH Key Location/Qualifiers
 FT Misc-difference 22 /note= "wild type Met replaced with Leu"
 FT FT Misc-difference 57 /note= "wild type Met replaced with Leu"
 FT
 XX
 PN MO9950398-A2.
 XX
 PD 07-OCT-1999.
 XX
 PF 26-MAR-1999; 99WO-US06641.
 XX
 PR 27-MAR-1998; 98US-0079751.
 XX
 PA (USSH) US DEPT HEALTH & HUMAN SERVICES.
 XX
 PI Newton DL, Rybak SM;
 XX
 DR WPI: 1999-610847/52.
 DR N-PSDB: AA208132.
 XX
 PT New recombinant ribonucleases, used for killing target cells, e.g. for
 PT treating cancers, viral infections or autoimmune diseases
 XX
 PS Claim 22; Page 64; 71pp; English.
 CC The present sequence is a recombinant Rana catesbeiana oocyte
 CC ribonuclease (RacOR1) protein with Met22Leu Met57Leu. Carboxy terminal

CC end of recombinant RacOR1 has a covalently bound ligand binding moiety,
 CC which can be a LL2 antibody directed against CD22 on cancerous B cells
 CC or human chorionic gonadotropin (hCG) effective against Kaposi's sarcoma
 CC cells. Recombinant ribonucleases can be expressed in bacteria without an
 CC N-terminal methionine due to the presence of a signal peptide that is
 CC cleaved by bacteria. The soluble expression of ribonuclease allows the
 CC proteins to be fused in-frame with ligand binding moieties to form
 CC cytotoxic fusion proteins. They can be used for treatment of cancer and
 CC autoimmune diseases.

XX Sequence 110 AA:
 SQ

Query Match 98.3%; Score 591; DB 20; Length 110;
 Best Local Similarity 98.2%; Pred. No. 4.3e-60;
 Matches 107; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

OY 2 NMATFOQKHINPIICNTIMDNIIYVGGCKRVNTFIISATYKATCTGVINNVLS 61
 DB 2 NMATFOQKHINPIICNTILDNIIYVGGCKRVNTFIISATYKATCTGVINNVLS 61
 OY 62 TTRFOLNCTRTSITPRPCPYSSRTETNYICVKCENQYVHFAIGRCR 110
 DB 62 TTRFOLNCTRTSITPRPCPYSSRTETNYICVKCENQYVHFAIGRCR 110

RESULT 6
 AAY28876
 ID AAY28876 standard; Protein; 111 AA.
 XX AAY28876;
 AC
 XX 25-JAN-2000 (first entry)
 DT
 XX
 DE Recombinant Met(-1) RacOR1 Met22Leu Met57Leu-(His)6 protein.
 XX
 DE Met(-1) Rana catesbeiana ribonuclease Metc22Leu Met57Leu-(His)6; RacOR1;
 KW recombinant; CD22; covalently bound; LL2 antibody; ligand binding moiety;
 KW cancerous B cell; Kaposi's sarcoma; human chorionic gonadotropin; hCG;
 KW signal peptide; recombinant ribonuclease; cytotoxic fusion protein;
 KW cancer; bullfrog; RNase; autoimmune disease.
 XX
 OS Rana catesbeiana.
 OS Synthetic.
 XX
 FH Key Location/Qualifiers
 FT Misc-difference 1 /note= "(His)6 histidine tag attached to N-terminal Met"
 FT Misc-difference 1 /note= "Met not found in wild type RacOR1"
 FT Misc-difference 23 /note= "Wild type Met replaced with Leu"
 FT Misc-difference 58 /note= "Wild type Met replaced with Leu"
 FT
 XX WO9950398-A2.
 PN
 XX 07-OCT-1999.
 PD
 XX 26-MAR-1999; 99WO-US06641.
 PF
 XX 27-MAR-1998; 98US-0079751.
 PR
 XX (USSH) US DEPT HEALTH & HUMAN SERVICES.
 PA
 XX Newton DL, Rybak SM;
 PI
 XX WPI: 1999-610847/52.
 DR N-PSDB; AAZ08133.
 XX
 PT New recombinant ribonucleases, used for killing target cells, e.g. for
 PT treating cancers, viral infections or autoimmune diseases
 XX
 PS Claim 22; Page 66; 71pp; English.

XX The present sequence is a recombinant Rana catesbeiana oocyte
 CC ribonuclease (RacOR1) protein with Met at position 1 attached to a
 CC (His)6 tag, Met23Leu and Met58Leu. Carboxy terminal end of recombinant
 CC RacOR1 has a covalently bound ligand binding moiety, which can be a LL2
 CC antibody directed against CD22 on cancerous B cells or human chorionic
 CC gonadotropin (hCG) effective against Kaposi's sarcoma cells. Recombinant
 CC ribonucleases can be expressed in bacteria without an N-terminal
 CC methionine due to the presence of a signal peptide that is cleaved by
 CC bacteria. The soluble expression of ribonuclease allows the proteins to
 CC be fused in-frame with ligand binding moieties to form cytotoxic fusion
 CC proteins. They can be used for treatment of cancer and autoimmune
 CC diseases.

XX Sequence 111 AA:
 SQ

Query Match 98.3%; Score 591; DB 20; Length 111;
 Best Local Similarity 98.2%; Pred. No. 4.4e-60;
 Matches 107; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

OY 2 NMATFOQKHINPIICNTIMDNIIYVGGCKRVNTFIISATYKATCTGVINNVLS 61
 DB 3 NMATFOQKHINPIICNTILDNIIYVGGCKRVNTFIISATYKATCTGVINNVLS 62
 OY 62 TTRFOLNCTRTSITPRPCPYSSRTETNYICVKCENQYVHFAIGRCR 110
 DB 63 TTRFOLNCTRTSITPRPCPYSSRTETNYICVKCENQYVHFAIGRCR 111

RESULT 7
 AAY33321
 ID AAY33321 standard; Protein; 111 AA.
 XX AAY33321;
 AC
 XX 29-NOV-1999 (first entry)
 DT
 XX
 DE Frog lectin protein fragment.
 XX
 DE Cytotoxic; RNase; ribonuclease; pancreatic; antibody; light chain;
 KW heavy chain; cell surface marker; treatment; tumor; viral infection;
 KW parasite infection; immune dysfunctional cell; autoimmune disease;
 KW contraceptive; cell separation; transplantation; bone marrow ablation;
 KW leukemia cell; T-cell; graft-versus-host disease; bullfrog; lectin.
 XX
 OS Rana catesbeiana.
 OS
 XX
 FH US955073-A.
 FT
 PN
 XX 21-SEP-1999.
 PD
 XX 09-JUL-1997; 97US-0891848.
 PF
 XX 22-SEP-1993; 93US-0125462.
 PR 22-OCT-1991; 91US-0779195.
 PR 20-APR-1990; 90US-0510696.
 PR 04-FEB-1993; 93US-0014082.
 PA
 XX (USSH) US DEPT HEALTH & HUMAN SERVICES.
 XX
 XX Rybak SM, Newton DL, Nicholls PJ, Youle RJ;
 PI
 XX WPI: 1999-560488/47.
 DR
 XX
 PT Recombinantly fused pancreatic RNase-targeting proteins useful for
 PT treating tumors, infections, immune or autoimmune disorders and as a
 PT contraceptive
 XX
 PS Example 3; Fig 19; 47pp; English.
 PS
 XX This invention describes a novel nucleic acid construct comprising
 CC sequences encoding functional pancreatic RNase and a second protein
 CC (preferably the light and heavy chains of an antibody) which binds a

CC specific cell surface marker on a target cell and functions as a
 CC cytotoxic agent. The products can be used for selectively killing cells
 CC expressing a specific surface marker. They can be used for treating
 CC tumors or infected cells (e.g. cells infected by viruses (especially
 CC latent or chronic virus infections, such as human immunodeficiency virus
 CC (HIV)-1, Epstein-Barr virus, herpes viruses (herpes simplex types 1 and
 CC II), hepatitis viruses (B, non-A-non-B, and delta), herpes zoster,
 CC cytomegalovirus) and cells infected with parasites (such as the malaria
 CC parasite)). They can also be used for treating immune dysfunctional cells
 CC in immune and autoimmune diseases. Additionally, they may be used as
 CC contraceptives. Finally they can also be used for cell separation in
 CC vitro by selectively killing unwanted types of cells (e.g. in bone
 CC marrow) prior to transplantation into a patient undergoing marrow
 CC ablation by radiation or for killing leukemia cells or T-cells that would
 CC cause graft-versus-host disease. This sequence represents a bullfrog
 CC (Rana catesbeiana) lectin used to describe the method of the invention.
 CC
 SQ Sequence 111 AA;
 Query Match 97.6%; Score 586.5; DB 20; Length 111;
 Best Local Similarity 99.1%; Pred. No. 1.4e-59;
 Matches 109; Conservative 0; Mismatches 0; Indels 1; Gaps 1;
 QY 2 NNATFOOKHIVNPII-CNTIMDNNTIYVGCKRVNFTIISATTVKATCGVIMNV 60
 DB 2 NNATFOOKHIVNPIIINCMNTIMDNNTIYVGCKRVNFTIISATTVKATCGVIMNV 61
 QY 61 STTRFQNLNCTRTSTTPRCPYSSRFTETNYICVCKENQYPVHFGIGRC 110
 DB 62 STTRFQNLNCTRTSTTPRCPYSSRFTETNYICVCKENQYPVHFGIGRC 111
 RESULT 8
 AAM06544
 ID AAM06544 standard; protein; 104 AA.
 AC AAM06544;
 DT 22-AUG-1997 (first entry)
 DE Antitumour protein from Rana pipiens oocytes.
 KW Tumour; chemotherapy; radiotherapy; frog.
 OS Rana pipiens.
 PN MO9639428-A1.
 PD 12-DEC-1996.
 PF 03-JUN-1996; 96WO-US08304.
 PR 06-JUN-1995; 95US-0467955.
 PA (ALFA-) ALFACELL CORP.
 PI Ardelit WJ;
 DR WPI; 1997-043063/04;
 PT Antitumour proteins from Rana pipiens oocyte(s) - have fewer
 PS disadvantages than chemotherapy, surgery and radiotherapy
 XX Claim 8; Page 28; 45pp; English.
 CC The present sequence is a specifically claimed example of an
 CC antitumour protein from the generic protein in AAM06544, with the
 CC molecular weight 12000. This is one of two preferred proteins (the
 CC other in AAM06544) that have been isolated from Rana pipiens oocytes.
 CC Both proteins have a blocked amino terminal group and are essentially
 CC free of carbohydrates. The proteins are used to treat tumours. Use of
 CC the peptides has fewer disadvantages than chemotherapy, radiotherapy
 CC and surgery in the treatment of tumours.

XX Sequence 104 AA;
 SQ Query Match 47.0%; Score 282.5; DB 18; Length 104;
 Best Local Similarity 50.0%; Pred. No. 1.1e-24;
 Matches 55; Conservative 15; Mismatches 31; Indels 9; Gaps 4;
 QY 2 NNATFOOKHIVNPII-CNTIMDNNTIYVGCKRVNFTIISATTVKATCGVIMNV 59
 DB 2 DMTFQOKHIVNPIIINCMNTIMDNNTIYVGCKRVNFTIISATTVKATCGVIMNV 57
 QY 60 LSTTRFQNLNCTRTSTTPRCPYSSRFTETNYICVCKENQYPVHFGIGRC 109
 DB 58 LSTTRFQNLNCTRTSTTPRCPYSSRFTETNYICVCKENQYPVHFGIGRC 104
 RESULT 9
 AAY28870
 ID AAY28870 standard; protein; 104 AA.
 AC AAY28870;
 DT 25-JAN-2000 (first entry)
 DE Recombinant RapaL1 GlnIser amino acid sequence.
 KW Recombinant Rana pipiens ribonuclease; RapaL1 GlnIser; covalently bound;
 KW LL2 antibody; ligand binding moiety; CD22; cancerous B cell; frog;
 KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
 KW recombinant ribonuclease; cytotoxic fusion protein; cancer; Rase;
 KW autoimmune disease.
 OS Rana pipiens.
 OS Synthetic.
 FH Key Location/Qualifiers
 FT Misc-difference 1 /note="Wild type Gln replaced with Ser"
 PN MO9950398-A2.
 PD 07-OCT-1999.
 PF 26-MAR-1999; 99WO-US06641.
 PR 27-MAR-1998; 98US-0079751.
 PA (USSH) US DEPT HEALTH & HUMAN SERVICES.
 PI Newton DL, Rybak SM;
 DR WPI; 1999-610847/52.
 DR N-PSDB; AA208128.
 PT New recombinant ribonucleases, used for killing target cells, e.g. for
 PS treating cancers, viral infections or autoimmune diseases -
 XX Claim 34; Page 60; 71pp; English.
 CC The present sequence is a recombinant Rana pipiens ribonuclease (RapaL1)
 CC protein with GlnIser. Carboxy terminal end of recombinant RapaL1 has a
 CC covalently bound ligand binding moiety, which can be a LL2 antibody
 CC directed against CD22 on cancerous B cells or human chorionic
 CC gonadotropin (hCG) effective against Kaposi's sarcoma cells. Recombinant
 CC ribonucleases can be expressed in bacteria without an N-terminal
 CC methionine due to the presence of a signal peptide that is cleaved by
 CC bacteria. The soluble expression of ribonuclease allows the proteins to
 CC be fused in-frame with ligand binding moieties to form cytotoxic fusion
 CC proteins. They can be used for treatment of cancer and autoimmune
 CC diseases.
 SQ Sequence 104 AA;

Query Match 46.7% Score 280.5; DB 20; Length 104;
 Best Local Similarity 49.5%; Pred. No. 1.8e-24;
 Matches 55; Conservative 15; Mismatches 32; Indels 9; Gaps 4;

OY 1 SMMATFOOKHILIN-PIICNTIMDNNIYIGGCKRVNTFLISSATYVKAICTGVI-NMN 58
 Db 1 SDWLTFQKKHLNTRDVCNNIMSTNLF---HCKDKNTFLYSRPEPVKAICKGIASKN 56

OY 59 VLASTTRQOLNCTRTSITPRCPYSSRTEETNYICVKCENQYPVHAFAGIGRC 109
 Db 57 VLTISEEYLSDC---NNTSRCKYKLLKKSNTNFCVTCENQAPVHAFVGVGHC 104

RESULT 10

AAV28871
 ID AAV28871 standard; Protein; 105 AA.

XX AAV28871;

XX 25-JAN-2000 (first entry)

DE Recombinant Met(-1) RapLRI GlnSer amino acid sequence.

XX Recombinant Met(-1) Rana pipiens ribonuclease GlnSer; RapLRI; CD22;
 KW covalently bound; LL2 antibody; ligand binding moiety; cancerous B cell;
 KW Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
 KW recombinant ribonuclease; cytotoxic fusion protein; cancer; frog;
 KW autoimmune disease; RNase.

OS Rana pipiens.
 OS Synthetic.

XX Key Location/Qualifiers

FT MISC-difference 1 /note= "Met not found in wild type RapLRI"

FT MISC-difference 2 /note= "Wild type Gln replaced with Ser"

FT W09950398-A2.

XX 07-OCT-1999.

XX 26-MAR-1999; 99W0-US06641.

XX 27-MAR-1998; 98US-0079751.

XX (USSH) US DEPT HEALTH & HUMAN SERVICES.

XX Newton DL, Rybak SM;

XX WPI; 1999-610847/52.

XX N-PSDB; AA208129.

XX New recombinant ribonucleases, used for killing target cells, e.g. for
 treating cancers, viral infections or autoimmune diseases

XX Claim 34; Page 61; 71pp; English.

XX The present sequence is a recombinant Rana pipiens ribonuclease (RapLRI)
 CC protein with Met at position 1 and GlnSer; Carboxy terminal end of
 CC recombinant RapLRI has a covalently bound ligand binding moiety, which
 CC can be a LL2 antibody directed against CD22 on cancerous B cells or human
 CC chorionic gonadotropin (hCG) effective against Kaposi's sarcoma cells.
 CC Recombinant ribonucleases can be expressed in bacteria without an N-
 CC terminal methionine due to the presence of a signal peptide that is
 CC cleaved by bacteria. The soluble expression of ribonuclease allows the
 CC proteins to be fused in-frame with ligand binding moieties to form
 CC cytotoxic fusion proteins. They can be used for treatment of cancer and
 CC autoimmune diseases.

SO Sequence 105 AA;

Query Match 46.7% Score 280.5; DB 20; Length 105;

Best Local Similarity 49.5%; Pred. No. 1.8e-24;
 Matches 55; Conservative 15; Mismatches 32; Indels 9; Gaps 4;

OY 1 SMMATFOOKHILIN-PIICNTIMDNNIYIGGCKRVNTFLISSATYVKAICTGVI-NMN 58
 Db 2 SDWLTFQKKHLNTRDVCNNIMSTNLF---HCKDKNTFLYSRPEPVKAICKGIASKN 57

OY 59 VLASTTRQOLNCTRTSITPRCPYSSRTEETNYICVKCENQYPVHAFAGIGRC 109
 Db 58 VLTISEEYLSDC---NNTSRCKYKLLKKSNTNFCVTCENQAPVHAFVGVGHC 105

RESULT 11

AAV28865
 ID AAV28865 standard; Protein; 104 AA.

XX AAV28865;

XX 25-JAN-2000 (first entry)

DE Rana pipiens liver ribonuclease (RapLRI).

XX Rana pipiens liver ribonuclease; RapLRI; covalently bound; LL2 antibody;
 KW ligand binding moiety; CD22; cancerous B cell; Kaposi's Sarcoma; frog;
 KW human chorionic gonadotropin; hCG; recombinant ribonuclease; RNase;
 KW signal peptide; cytotoxic fusion protein; cancer; autoimmune disease.

OS Rana pipiens.

XX W09950398-A2.

XX 07-OCT-1999.

XX 26-MAR-1999; 99W0-US06641.

XX 27-MAR-1998; 98US-0079751.

XX (USSH) US DEPT HEALTH & HUMAN SERVICES.

XX Newton DL, Rybak SM;

XX WPI; 1999-610847/52.

XX N-PSDB; AA208124.

XX New recombinant ribonucleases, used for killing target cells, e.g. for
 treating cancers, viral infections or autoimmune diseases

XX Claim 1; Page 55; 71pp; English.

XX The present sequence is Rana pipiens liver ribonuclease (RapLRI)
 CC protein. Carboxy terminal end of RapLRI has a covalently bound
 CC ligand binding moiety, which can be a LL2 antibody directed against
 CC CD22 on cancerous B cells or human chorionic gonadotropin (hCG)
 CC effective against Kaposi's Sarcoma cells. Recombinant ribonucleases can
 CC be expressed in bacteria without an N-terminal methionine due to the
 CC presence of a signal peptide that is cleaved by bacteria. The soluble
 CC expression of ribonuclease allows the proteins to be fused in-frame with
 CC ligand binding moieties to form cytotoxic fusion proteins. They can be
 CC used for treatment of cancer and autoimmune diseases.

SO Sequence 104 AA;

Query Match 46.0% Score 276.5; DB 20; Length 104;
 Best Local Similarity 49.1%; Pred. No. 5.2e-24;
 Matches 54; Conservative 15; Mismatches 32; Indels 9; Gaps 4;

OY 2 SMMATFOOKHILIN-PIICNTIMDNNIYIGGCKRVNTFLISSATYVKAICTGVI-NMN 59
 Db 2 DWLTFQKKHLNTRDVCNNIMSTNLF---HCKDKNTFLYSRPEPVKAICKGIASKN 57

OY 60 LSTTRQOLNCTRTSITPRCPYSSRTEETNYICVKCENQYPVHAFAGIGRC 109
 Db 58 LTTSEYLSDC---NNTSRCKYKLLKKSNTNFCVTCENQAPVHAFVGVGHC 104

RESULT 12

AAV28867
ID AAV28867 standard; Protein: 105 AA.

AAV28867;

25-JAN-2000 (first entry)

Recombinant Met(-1) RapLRI.

Recombinant Met(-1) Rana pipiens ribonuclease; RapLRI; CD22; RNase;

covalently bound; LL2 antibody; ligand binding moiety; cancerous B cell;

Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;

recombinant ribonuclease; cytotoxic fusion protein; cancer; frog;

autoimmune disease;

Rana pipiens.

Synthetic.

Key

Misc-difference 1

Location/Qualifiers

/note="Met not found in wild type RapLRI"

MO9950398-A2.

07-OCT-1999.

26-MAR-1999; 99WO-US06641.

27-MAR-1998; 98US-0079751.

(USSH) US DEPT HEALTH & HUMAN SERVICES.

Newton DL, Rybak SM;

WPI: 1999-610647/5;

N-PSDB; AAZ08126.

New recombinant ribonucleases, used for killing target cells, e.g. for

treating cancers, viral infections or autoimmune diseases

Claim 34; Page 57; 71pp; English.

The present sequence is a recombinant Rana pipiens ribonuclease (RapLRI)

protein with Met at position 1. Carboxy terminal end of recombinant

RapLRI has a covalently bound ligand binding moiety, which can be a LL2

antibody directed against CD22 on cancerous B cells or human chorionic

gonadotropin (hCG) effective against Kaposi's sarcoma cells. Recombinant

ribonucleases can be expressed in bacteria without an N-terminal

methionine due to the presence of a signal peptide that is cleaved by

bacteria. The soluble expression of ribonuclease allows the proteins to

be fused in-frame with ligand binding moieties to form cytotoxic fusion

proteins. They can be used for treatment of cancer and autoimmune

diseases.

AAW35118
ID AAW35118 standard; Protein: 112 AA.

AAW35118;

20-APR-1998 (first entry)

R. pipiens recombinant RNase protein NLSmeterronc.

RNase A; ribonuclease; cytotoxic; onconase; nOnc; immunofusion;

tumour cell growth; frog.

Rana pipiens.

MO9731116-A2.

28-AUG-1997.

19-FEB-1997; 97WO-US02588.

21-FEB-1996; 96US-0011800.

(USSH) US DEPT HEALTH & HUMAN SERVICES.

Boque L, Newton DL, Rybak SM, Wlodawer A;

WPI: 1997-435168/40.

N-PSDB; AAT94955.

Ribonuclease molecules based on native Onconase - used for killing

cells, particularly tumour cells

Claim 18; Page 63; 90pp; English.

AAW35115 to AAW35123 encode recombinant proteins (rOnc) which are

modifications of the RNase Onconase (RNase). Such novel

ribonuclease molecules are highly cytotoxic and can be used alone or to

form chemical conjugates or to target recombinant immunofusions. They are

used particularly for decreasing tumour cell growth. They can also be

used for cell separation in vitro by selectively killing unwanted types

of cells, e.g. in bone marrow prior to transplantation into a patient

undergoing marrow ablation by radiation, or for killing leukaemia cells

or T-cells that would cause graft versus host disease. The toxins can

also be used to selectively kill unwanted cells in culture. The new

ribonucleases have increased cytotoxic activity compared to nOnc and also

lower immunogenicity in humans.

Sequence 112 AA;

Query Match

Best Local Similarity 46.0%; Score 276.5; DB 18; Length 112;

Matches 55; Conservative 15; Mismatches 32; Indels 9; Gaps 4;

1 SNAATFOOKHINT-PIICNTIMDNNTIYVGCGCKRVNFTIISATYVKAICTGVI-NMN 58

9 SDWITFOKHINTNRDVCNNIMSTNLF---HCKDKNTFTIISPEPYKAICKITASKN 64

59 VLTSTFEPYLSDC---NWTSPRCATYKTKSTNFCVTCENQAPVHFVGSC 112

RESULT 14

AAV28879
ID AAV28879 standard; Protein: 127 AA.

AAV28879;

25-JAN-2000 (first entry)

Rana pipiens Clone 5a1b ribonuclease.

Rana pipiens ribonuclease Clone 5a1b; RapLRI; covalently bound; RNase;

tumour cell growth; frog.

Rana pipiens.

MO9731116-A2.

28-AUG-1997.

19-FEB-1997; 97WO-US02588.

21-FEB-1996; 96US-0011800.

(USSH) US DEPT HEALTH & HUMAN SERVICES.

Boque L, Newton DL, Rybak SM, Wlodawer A;

WPI: 1997-435168/40.

N-PSDB; AAT94955.

Ribonuclease molecules based on native Onconase - used for killing

cells, particularly tumour cells

Claim 18; Page 63; 90pp; English.

AAW35115 to AAW35123 encode recombinant proteins (rOnc) which are

modifications of the RNase Onconase (RNase). Such novel

ribonuclease molecules are highly cytotoxic and can be used alone or to

form chemical conjugates or to target recombinant immunofusions. They are

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used for cell separation in vitro by selectively killing unwanted types

of cells, e.g. in bone marrow prior to transplantation into a patient

undergoing marrow ablation by radiation, or for killing leukaemia cells

or T-cells that would cause graft versus host disease. The toxins can

also be used to selectively kill unwanted cells in culture. The new

ribonucleases have increased cytotoxic activity compared to nOnc and also

lower immunogenicity in humans.

Sequence 112 AA;

Query Match

Best Local Similarity 49.5%; Score 276.5; DB 18; Length 112;

Matches 55; Conservative 15; Mismatches 32; Indels 9; Gaps 4;

1 SNAATFOOKHINT-PIICNTIMDNNTIYVGCGCKRVNFTIISATYVKAICTGVI-NMN 58

9 SDWITFOKHINTNRDVCNNIMSTNLF---HCKDKNTFTIISPEPYKAICKITASKN 64

59 VLTSTFEPYLSDC---NWTSPRCATYKTKSTNFCVTCENQAPVHFVGSC 112

